BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problems Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C12M 3/04, 1/24, 1/04 — A1

(11) International Publication Number: WO 90/02171

(43) International Publication Date: 8 March 1990 (08.03.90)

(21) International Application Number: PCT/US89/03644

(22) International Filing Date: 28 August 1989 (28.08.89)

(30) Priority data:
238,445 31 August 1988 (31.08.88) US

(71) Applicant: CELLCO ADVANCED BIOREACTORS, INC. [US/US]; 5516 Nicholson Lane, Kensington, MD 20895 (US).

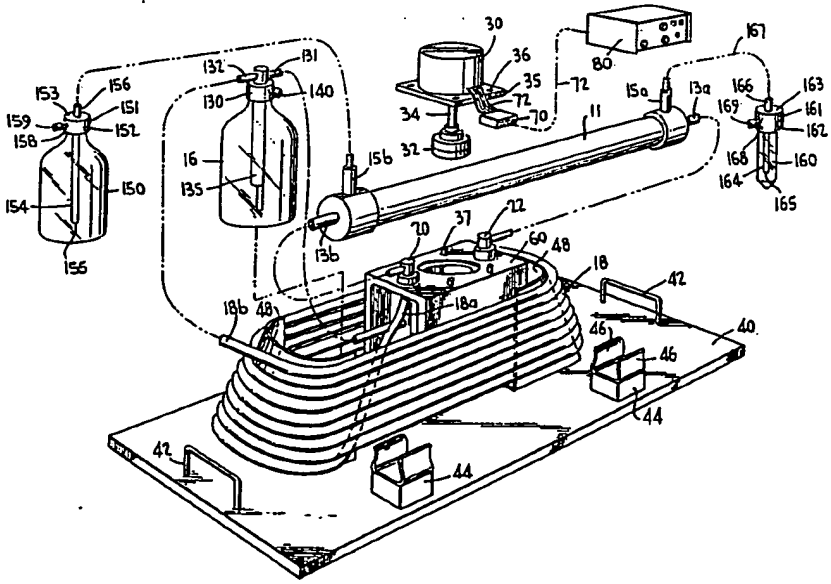
(72) Inventors: KNAZEK, Richard, A. ; 9424 Locust Hill Road, Bethesda, MD 20814 (US). KIDWELL, William, R. ; 10905 Lowell Court, Ijamsville, MD 21254 (US). SCOTT, Stephen, C. ; 1702 Dogwood Drive, Frederick, MD 21201 (US). HANLEY, Matthew, W. ; 1027 C Street, N.E., Washington, DC 20003 (US).

(74) Agents: STERN, Marvin, R. et al.; Fleit, Jacobson, Cohn, Price, Holman & Stern, The Jenifer Building, 400 Seventh Street, N.W., Washington, DC 20004 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: IN VITRO CELL CULTURE REACTOR



(57) Abstract

A compact in vitro cell culture reactor (10) drives a liquid nutrient media drawn from a standard cell culture bottle reservoir (16) with a sealed pump (24), through intracapillary space of a cell culture cartridge (11), through a length of silicone rubber tubing (18) coiled in an ascending helix and serving as a passive oxygenator, and return to reservoir (16). A motor controller (80) drives a pump motor (30) magnetically coupled to pump (24) through independently adjustable duty cycles to periodically vary perfusate circulation in opposite directions through the perfusate circuit. Cartridge (11) is fitted with quick disconnecting couplers (20, 22) to enable remove and substitution of other cartridges. Cell products may be harvested from extracapillary space of cartridge (11) via side ports (15a, 15b).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic			SN	Senegal
CG	Congo	KR	Republic of Korea	SU	Soviet Union
CH	Switzerland	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany, Federal Republic of	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

IN VITRO CELL CULTURE REACTOR

TECHNICAL FIELD

This invention relates to the growth of living cells and, more particularly, to methods and apparatus for growing cells on semi-permeable, tube-shaped membranes (hereinafter referred to as "capillaries") and for harvesting products from growing cells. Cells may be defined as either adhering to the capillaries, and as being either prokaryotic or eukaryotic (i.e., bacterial, yeast, plant or animal).

BACKGROUND ART

As explained in U.S. Patent No. 3,883,393 and its parent, U.S. Patent No. 3,821,087, of Knazek, et al., both of which references are hereby incorporated herein by reference, cells suspended in a nutrient medium are initially allowed to settle onto the outer surfaces of capillaries which are continuously perfused by oxygenated nutrient medium flowing through the capillaries. Nutrient substances diffuse from the perfusing medium through the capillary walls and into the cells, while cell products such as lactic acid and hormones, diffuse from the cell through the capillary walls into the perfusate from which these products may be recovered.

Attempts to grow cells to densities or with structures approaching those of living tissues have included various means of supplying nutrient medium to the cells. Very high cell densities have been obtained, for example, in suspension cultures, and three dimensional growth of tumor cells in thin layers has been induced. The techniques and apparatus heretofore available for cell culture as, for example, taught in the U.S. Patent Nos. 3,821,087 and 3,883,393 have been particularly suited to large scale processes

using large, multi-cartridge units with several cell culture cartridges arranged in parallel. Consequently, both the large-scale nature of the process and its novelty resulted in complex, expensive apparatus capable of providing continuous, on-line measurement of process parameters, as well as a separate pump head for each cell culture unit to assure identical flow rates through each unit.

Moreover, cell growth during start-up of heretofore available apparatus, as well as slow transient periods of cell growth following replacement of individual cartridges have compromised the productivity of the apparatus. Additionally, the size, initial expense and operator manpower requisite to operation and monitoring have made currently available apparatus too expensive and unsuitable for small laboratories.

STATEMENT OF THE INVENTION

Accordingly, it is an object of the present invention to provide an improved method and reactor for in-vitro cell culture growth.

It is another object to provide a method and reactor for in-vitro cell culture growth suitable for small-scale and start-up operations.

It is yet another object to provide a simplified method and reactor for in-vitro cell culture growth.

It is still another object to provide a compact reactor for in-vitro cell culture growth.

It is still yet another object to provide an inexpensive process and reactor for in-vitro cell culture growth.

It is a further object to provide a process and reactor using a simplified passive oxygenator

enhancing oxygen and carbon dioxide gas transfer during in-vitro cell culture growth.

It is a yet further object to provide a process and reactor facilitating removal and replacement of cell culture cartridges during in-vitro cell culture growth.

It is a still further object to provide a process and reactor enhancing productivity during in-vitro cell culture growth.

It is still yet another object to provide a process and reactor facilitating retrieval of cells and cell products from an in-vitro cell culture growth process.

These and other objects are achieved in a process and with a reactor using a standard tissue culture medium bottle as a reservoir for nutrient media supplied to a perfusion circuit, a length of tubing of a silicone material permeable by oxygen and carbon dioxide gases serving as an oxygenator for the perfusion circuit, a removeable cell culture cartridge including a shell connectable into the perfusion circuit via spaced end portions, and a plurality of capillaries having walls permeable to nutrients in the liquid media, to cell products of a large molecular weight and to gases extending the perfusion circuit between the spaced end portions with the capillaries dividing a chamber within the shell into intracapillary space and extracapillary space, and a sealed pump connectable between the reservoir and one of the end portions of the cartridge completing the perfusion circuit. A motor controller periodically enhances transport of nutrients and gases through the intracapillary space by controlling operation of the pump to provide independently variable duty cycles

during distinct forward direction phase and reversed direction phase of perfusate circulation through the circuit. The reactor may be mounted on a hand-holdable rigid base retaining the reservoir, pump and cell culture unit in an ordered, alterably connected configuration of the perfusion circuit with the silicone tubing of the oxygenator supported in a serially wound helical coil surrounding the pump and reservoir while supported by spaced apart perforated racks transversely extending from the base. A plurality of clips attached to the base enables the cell culture cartridge to be mounted in an aligned array spaced apart from the racks, along one side of the base while exposed to direct visual operator observation.

BRIEF DESCRIPTION OF THE DRAWING

A more complete appreciation of this invention, and many of the attendant advantages thereof, will be readily apparent as the same becomes better understood by reference to the following detailed description when considered in conjunction with the accompanying drawing in which like reference symbols indicate the same or similar components wherein:

Figure 1 is an exploded orthogonal projection showing interconnections of the several components of a cell culture reactor according to the principles of the present invention;

Figure 1A is a schematic diagram illustrating interconnections between the several component elements of the cell culture reactor of Figure 1;

Figure 2 shows one side view of the cell culture reactor of Figure 2;

Figure 3 is a partially cut-away sectional view of the cell culture reactor of Figure 2;

Figure 4 is an exploded, partially cut away side view of a connector suitable for use in a cell culture reactor according to the principles of the present invention;

Figure 5 is a top sectional view of the female element of the connector taken along line 5-5' in Figure 4;

Figure 6 is a side, partially cut-away view of an assembled connector;

Figure 7 is a small scale view showing a physical arrangement of electrical connections between the reactor of Figure 1 and a controller;

Figure 8 is a front view showing a control panel layout for a controller useable with the reactor of Figure 1;

Figure 9 is a cross-sectional view of a cell culture unit;

Figure 10 is an orthogonal projection showing details of oxygenator racks;

Figure 11 is a partially cut-away side view showing structure of a cap for a liquid nutrient media reservoir;

Figure 12 is an electrical schematic diagram of timer stages for a controller;

Figure 13 is an electrical schematic diagram of a rectifier stage for the controller; and

Figure 14 is a two coordinate graph showing molecular weight as a function of sieving coefficients for three types of DEAE-cellulose cartridges.

DETAILED DESCRIPTION OF THE INVENTION

Referring to Figures 1, 2 and 3, reactor apparatus 10 enables a process for growing cell

cultures on capillaries and for harvesting cells, and cell products (e.g., living tissue or excretory products) from the capillaries. Reactor 10 includes a cell culture unit 11 containing a plurality of aligned, parallel capillaries 12 constructed of semi-permeable material. Referring briefly to Figure 9, a plurality of capillaries 12 are preferably present in each cell culture unit 11, forming a bundle of capillaries to provide a system simulating a vascular network of living tissue. Capillaries 12, typically having a length of about ten to twelve inches, are inserted into the unit's shell 13 formed of inert plastic material such as polycarbonate, and the ends of capillaries 12 are secured in end pieces 14 formed of epoxy resin or other suitable sealing material at each end of shell 13 so that a liquid nutrient medium flowing into an end cap 13a of cell culture 11 will pass through capillaries 12 and exit through the opposite end cap 13b of unit 11, as is more particularly described in the aforementioned U.S. Patent Numbers 3,821,087 and 3,883,393. Thus, a nutrient medium introduced on the shell side of capillaries 12 will experience reduced bulk mixing with the perfusate. Separate perfusion of the shell side through ports 15a, 15b with reduced bulk mixing with the perfusate flowing through the capillaries is also possible. It should be noted that as used herein, the word "nutrient" includes gases such as oxygen.

A reservoir 16, such as a standard tissue culture medium bottle, serves as a reservoir to minimize the work involved in re-feeding cell culture within unit 11. A helical coil of tubing 18 made from a commercially available, medical grade non-cytotoxic material that is permeable to gases such as oxygen and

carbon dioxide, such as silicone rubber enables both oxygen and carbon dioxide gases to diffuse into perfusion medium circulating through the perfusion circuit, thereby providing oxygenation and pH control of the perfusate. As may be seen from the schematic diagram of Figure IA, the upper end of tubing 18 is connected to one side of reservoir 16, while the lower end of tubing 18 is coupled to the male element of a quick disconnecting coupler 20 which, in turn, has its female side coupled to opposite end cap 13b of cell culture unit 11. End cap 13a is coupled to a male element of a quick disconnecting coupler 22. A pump 24 with its input port 26 coupled to a second side of reservoir 16, discharges perfusate via its output port 28 through the female element of coupler 22. A bottle having the capacity of about 600 milliliters has been used as reservoir 16.

Reactor 10 is mounted on a rectangular, stainless steel tray 40 having easily graspable handles 42 at either end, enabling an operator to easily lift and place the reactor in either an incubator or a steam autoclave. A pair of clips 44 are positioned in axial alignment along one side of tray 40 to receive, and retain cell culture cartridge 11. The sides 46 of clips 44 are inwardly biased and are spaced apart by a distance slightly less than the diameter of cartridge 11, thereby assuring that the cartridge will be retentively held along the one side of tray 40 in full visual display for a human operator. A pair of spaced apart racks 48 engage and extend transversely from the surface of tray 40. As shown in greater detail in Figure 10, each rack 48 includes a plurality of vertically aligned, outwardly extending tabs 49 which support non-cytotoxic, gas permeable tubing 18 in the

ascending, serially wound helical coil shown in Figures 1, 2 and 3. In addition to holes 50 adjacent tabs 49, a plurality of additional holes 51 perforate the sides of both racks 48, thereby enhancing the exposure of non-cytotoxic, gas permeable tubing 18 to an ambient carbon dioxide environment within an incubator.

Pump 24 is held by a housing 60 made of an inert material such as, for example polypropylene, in position relative to tray 40 with inlet and outlet ports 26, 28 extending through housing 60. Pump 24 includes a motor 30 driving a circular magnetic coupler 32 via a shaft 34. Motor 30 is mounted on a square plate 35 perforated by four mounting holes 36. Four alignment pins 37 extending vertically above an upper horizontal surface of housing 60 fit through holes 36 and position motor 30 and its coupler 32, the driving components of the pump, in co-axial alignment with the driven components 24 of the pump, namely a ferromagnetic element 27 and rotor 29 encased within the sealed housing 31 of pump 24. Rotor 29 is made of Ryton, entirely encased within casing 31, and circulates perfusate between ports 26, 28 thereby causing perfusate to transverse the "perfusate circuit" of reactor 10. Port 28 is coupled directly to the female element of coupler 22 while port 26 is coupled to one side of reservoir 16. Motor 30 is electrically plug coupled (via plug-jack pair 70 and a multi-lead ribbon cable 72) to a programmable controller 80, as may be seen with greater particularity by reference to Figure 7, and is held in engagement with pump 24 principally by magnetic attraction arising from a completed magnetic circuit between the circular magnet of coupler 32 and ferromagnetic element 27 of the driven pump 24, and secondarily by gravity and, to a

lesser extent, by a friction-fit engagement of a lower circular flange 35a of plate 35 with a conforming circular recess 60a of housing 60. Consequently, motor 30 is accorded one degree of freedom to rotate pump 24 either clockwise or counterclockwise, but can be easily removed to enable the remainder of reactor apparatus 10 to be autoclaved, simply by grasping and lifting the exterior of motor 30 vertically upward, co-axially with shaft 34, thereby disengaging coupler 32 from element 27.

Referring now to Figures 4, 5 and 6, quick disconnecting couplers 20, 22 are substantially identical commercially available items each having a male element 90 co-axially insertable into a mating female element 91. Male element 90 includes an exterior port 92 disposed at right angles to an interior port 93. Interior port 93 is formed by an axially reciprocating sleeve 94 biased to protrude outwardly by a spring (not shown) mounted inside an outer sleeve 95. An O-ring 96 is seated within a circumferential groove in outer sleeve 95, to form an air-tight sealing relation with the interior element 91.

Element 91 includes a central lumen 97 communicating with an exterior port 98. A receiver 99 having exterior cross-sectional dimensions conforming with those of lumen 97, is disposed within lumen 97 to axially reciprocate and to receive inner sleeve 94 while an upper flange 102 seats against a junction 103 formed between inner sleeve 94 and outer sleeve 95 as element 90 is forced within lumen 97. As shown in Figure 6, when upper flange 102 engages junction 103 as element 90 is fully inserted within lumen 97, a lower flange 104 seats, in an air-tight manner against an

external shoulder 105 of element 91, to provide a continuous air-tight passage extending between exterior port 92 and exterior port 98.

Referring again to Figures 4, 5 and 6, a finger operable slide 106 has a major surface 107 disposed transversely to the longitudinal axes IC8 of elements 90, 91. A readily disposed spring 109 urges slide 106 to a radially eccentric position displaced from axes 108. An axially disposed spring 110 biases a detent 111 to extend axially upwardly, as shown in Figure 4, with the wider part 112 of the upper neck of detent 111 extending through the wider portion 113 of a slot in surface 107. As element 90 is forced within lumen 97, a shoulder 114 of element 90 engages a wide terminal end 115 of detent 111, thereby forcing detent 111 axially downwardly, and enabling radially disposed spring 109 to force slide 106 radially outwardly (i.e., to the right as shown in Figures 4, 5 and 6); consequently, a narrower portion 116 of the slot in surface 107 receives a smaller diameter portion 117 of detent 111, thus accommodating the radial displacement of slide 106. The radial displacement of slide 106 causes the inward periphery 118 of surface 107 to engage a recess 119 in element 90, thereby locking element 90 into lumen 97 with a continuous, air-tight passage extending between ports 92, 98.

Referring now to Figures 1 through 3 and particularly to Figure 11, a cap 130 closes the sole opening of reservoir 16. Cap 130 includes a first port 131 connectable to intake port 26 of pump 24 and a second port 132 directly connectable to upper end 18b of tubing 18. Cap 130 is formed with a skirt 133 open at its lower end and at its upper end closed by a base 134. An elongated rod 135 extends through base 134,

and is spaced inwardly apart from skirt 133, in a nearly co-axially arrangement. Rod 135 includes a first and longer lumen 136 connecting port 131 with the interior of reservoir 16, and a second, and shorter lumen 137 connecting port 132 with the interior of reservoir 16. As shown in Figure 11, a short rod 138 may be mounted upon the distant end of rod 135 to extend lumen 136 vertically downward, beyond the distal end of lumen 137, thereby facilitating manufacture of cap 130.

Skirt 133 includes female threads for engaging corresponding threads typically found on the exterior of the neck surrounding the sole opening of a container such as reservoir 16. When threadingly engaged with the neck of reservoir 16, skirt 133 and base 134 compress a gasket I30 disposed within cap 130, adjacent the interior surface of base 134, against the uppermost, exterior lip of the neck of reservoir 16, thereby with the exception of ports and lumina extending through base 134, forming an air-tight sealing engagement between cap 130 and reservoir 16. A third port 139 extends through skirt 133 and base 134, and into the upper interior of reservoir 16. A threadingly engaging plug 140 may be used to close port 139.

Rigid rod 135 with the two lumina 136, 137 enable these lumina to be sterilely inserted through the neck of reservoir 16 without the necessity for a human operator to either grasp, touch or otherwise guide the rod, as would be the case if lumina 136, 137 were simply sections of flexible tubing extending from upper end 18b and inlet 26 through base 134. Consequently, reservoir 16 may be easily coupled and removed from a perfusate circuit of reactor 10 simply

by firmly grasping cap 130 while turning reservoir 16 without either contaminating rod 135 or being contaminated by the contents of reservoir 16.

Referring again to Figure 1, a second reservoir 150 having a cap 151 constructed similarly to cap 130, albeit with a skirt 152 having its upper end closed by a base 153 perforated by an elongated rod 154 containing a single lumen 155 extending through base 153, couples the interior of reservoir 150 with an exterior port 156. Exterior port 156 may be, in turn, coupled with one-quarter inch diameter silicone tubing, for example, to a side port 15b of end cap I3b, thereby coupling the interior of reservoir 150 with the extracapillary side of cell culture cartridge 11. Alternatively, tubing 157 may extend directly through base 153 and into the interior of reservoir 150, thereby replacing rod 154 and its lumen 155.

A third reservoir 160 having a cap 161 threadingly engaging its upper neck, may be coupled with an additional length of one-quarter inch silicone tubing 167 to side port 15a of end cap 13a to communicate directly with the extracapillary space of cartridge albeit at a distance separated from side port 15b by a majority, if not the entire length of the extracapillary chamber of cartridge 11. Cap 161 may be constructed with a skirt 162 closed at its upper end by base 163 perforated by a rod 164 containing a single lumen 165 connecting an exterior port 166 with the interior of the container forming third reservoir 160. Alternatively, one end of tubing 167 may extend directly through in an air-type manner, base 163 and communicate directly with the interior of third reservoir 160.

Both caps 151, 161 contain a side port 158, 168 extending through skirts 152, 162 and communicating directly with the upper portions of the interiors of reservoirs 150, 160. Side ports 158, 168 may be closed with plugs 559, 169, respectively.

When port 150 is coupled to either side port 15a or 15b, with port 166 coupled to the other side port 15b, 15a. a source of pressure may be applied to the interior of reservoir 150 via side port 158, with tubing clamps (not shown) closing tubing connections to end caps 13a, 13b, so additional cells may be forced from reservoir 150 under pressure and into the extracapillary chamber of cartridge 11 while grown cells are harvested from the extracapillary chamber via side port 15a and received into reservoir 160. Upon completion, and re-filling of the extracapillary space, removal of the tubing clamps from the end caps and re-starting of perfusion circulation, reactor 10 may be replaced into a carbon dioxide incubator to resume cell growth.

Referring now to Figure 8, controller 80 includes three switches 180-182 and three dial adjustment potentiometers 183-185. Switch 180 is a single throw device for connecting power to controller 80; an indicator light D5 is illuminated when switch 180 is in an "on" position. Referring now to Figure 12 in conjunction with Figure 8, switch 181 is a three position, three pole device coupling a 12 volt source to either a "forward" terminal A or to a "reverse" terminal B of a multiplexer chip 188. When in the "auto" position, switch 181 interrupts application of the 12 volt source to either the A or the B terminal of multiplexer 188.

The timer stages shown in Figure 12 include two programmable timers 190, 191 (typically a number 4541 monolithic integrated circuit chip). Each timer has its own, discrete duty cycle adjustment with dial potentiometers 183, 184 serially connected with fixed resistors R1, R2, respectively to independently adjust the duration of duty cycles of pump motor 30 via timers 190, 191. Potentiometers 183, I84 are coupled by resistors R1, R2 to terminal #1 of timers 190, 191, in parallel with capacitors C1, C2 and fixed resistors R3, R4, respectively. Resistors R1, R2 set the minimum time for each duty cycle while potentiometers 183, 184 with dials mounted on the exterior control panel of controller 80 adjust the duty cycle from values exceeding the minimum times set by resistors R1, R2.

Reset terminal #6 of timer 190 is coupled via switch 182 when in its "start/reset" position to a 12 volt source, thereby applying a "reset" pulse to terminal #6 and initiating a timing sequence. Switch 182 is biased to toggle from the "start/reset" position to its "run" position when released by an operator thereby interrupting application of the 12 volt source to reset terminal #6. Timer 190 responds to the 12 volt reset signal applied to terminal #6 by generating an output signal at terminal #8 which is inverted by inverter 192 to provide an output signal exhibiting a low level logic state during the duty cycle established by the adjustment stage including resistor R1 and potentiometer 183. The inverted signal from inverter 192 is applied to terminal C of multiplexer 188 which, in turn, applies a control signal via inverters 193, 194 to pin #8 of a commercially available motor controller to determine the direction of pump rotation. Assuming a convention that a low level logic

signal from inverter 192 causes clockwise rotation of rotor 29, thereby providing a forward direction circulatiOn of perfusate from port 26 to port 28 in the perfusate circuit, then timer 190 controls the duration Of the first phase duty cycle during forward direction circulation of perfusate, while timer 191 controls the duration of the second phase duty cycle during reversed direction circulation.

At the end of its duty cycle as determined by potentiometer 183 and resistor R1, timer 190 generates a low logic state output signal which, after inversion by inverter 192, applies a high logic state signal to terminal C of multiplexer 188 and a reset pulse via capacitor C4 to reset terminal #6 of timer 191, thereby initiating a second phase of perfusate circulation with timer 191 providing a high logic state output signal via its #8 terminal, for the duration of its duty cycle as determined by resistor R2 and potentiometer 184. When inverted by inverter 195, the output signal from the timer 191 is at a low logic state and is coupled by a capacitor C4 to reset terminal #6 of timer 190. Consequently, during the duration of the second phase, that is during the duration of the duty cycle established by resistor R2 and potentiometer 184, timer 190 continues to exhibit a low logic state signal to inverter 192 which, in turn, continues to apply a high logic state signal to terminal C of multiplexer 188. Upon expiration of the second duty cycle, timer 191 generates a low logic state signal inverted by inverter 191 to apply a high logic state signal by a capacitor C4 to reset terminal #6 of timer 190, thereby terminating the second phase of perfusate circulation (i.e., counter-clockwise pump rotation with perfusate circulating from outlet port 28 through pump 24 and

being discharged via outlet port 26), and triggering a resetting of timer 190 to initiate repetition of the first phase of perfusate circulation during the first duty cycle established by resistor R1 and potentiometer 183. Light emitting diodes D1, D2 coupled between inverters 193, 194 and between output terminal #13 of multiplexer 188 and inverter 193, and a reference potential such as the circuit ground, may be mounted on the control panel of controller 80 adjacent dials 183, 184 respectively to indicate the phase of operation of pump motor 30 and the direction of perfusate circulation.

The motor controller (not shown) may be a commercially available item such as a model number BLD224-5 Vexta controller circuit manufactured by Oriental Motor Co., Ltd. Terminal connections of the Vexta circuit are shown in the following Table 1:

TABLE 1

PIN NUMBER	FUNCTION
1	Positive D.C. Voltage
2	Negative D.C. Voltage (at a ground potential)
5	Speed Control
6	Speed Control
7	Speed Control
8	Pump Direction
9	Pump Start/Stop

A separate potentiometer 185 may be coupled to pin numbers 5, 6 and 7 of a controller such as the Vexta BLD224-5 to control the speed of pump 24 and thus the relative flow of perfusata through the pump independently of the direction of perfusate flow.

When switch 180 initially applies power to controller 80, pump motor 30 is held in a stopped condition bY applicatiOn of a low logic state signal to pin number 9 of the Vexta controller because diodes D6, D7 function as an or gate controlling the "reset" terminal of latch 197, with capacitor C7 and resistance R5 serving to power-up latch 197 to its reset mode upon initial energization of the network (i.e., with application of a positive twelve volts to R5 and C7 initially discharged). Capacitors C5, C6, and their circuit reference potential coupling resistors R6, R7 and R8, R9 serve as integrators of signals applied to the "set" and reset terminals of latch 197 via switch 182. Subsequently, toggling of switch 182 to its "start/reset" position applies a positive 12 volt pulse via capacitor C5 to inverter 196, thus applying a negative pulse to the set electrode #3 of latch 197, thus causing the latch to apply a high logic state output signal to pin number 9. Simultaneously, a light emitting diode D3 coupled between terminal #1S of latch 197 and a circuit reference potential is lit, thereby indicating a "pump on" condition. Alternatively, when switch 182 is manually placed in its "stand-by" position, a positive 12 volt pulse is applied to inverter 198 and, as an inverted pulse, to the reset terminal #4 of latch 197, thereby causing the latch apply a low logic state signal to pin number 9 of the Vexta controller, thereby interrupting pump motor 30 and perfusate circulation, while simultaneously next extinguishing diode D3.

The rectifier circuit shown in Figure 13 includes a transformer T1 with a pair of primary windings and taps to accommodate various wall socket, alternating voltages available worldwide. A center

tapped secondary winding of transformer TI is coupled across a full wave rectifier chip 200 having its negative node coupled to the network reference potential. The positive node of rectifier 200 is taken via a voltage controller 2D2 (a LM350T) to apply a positive 24 volt to pin 1 of the Vexta controller relative to the negative node of rectifier 200 applied to pin number 2. A center tap from the secondary winding of transformer T1 is rectified with diode D4 and filtered to provide a positive 12 volt source for the timer circuit shown in Figure 12. Voltage regulator 204 (an AN 7812) maintains the twelve volt amplitude. A light emitting diode D5 coupled between the 12 volt source and the network reference potential is illuminated upon application of a voltage source across a primary winding of transformer T1, to indicate that switch 180 is in an "on" position with power applied to controller 80.

Reactor 10 provides a compact, easy to use hollow fiber cell culture system designed for use in laboratory-scale or start-up operations. All components of the reactor attacking either cells or nutrient medium should be made of Food and Drug Administration approved materials. The reactor is intended for use in a humidified air and carbon dioxide incubator and, through controller 80, provides automatic reversal of perfusate flow and control of perfusate flow rate.

Reservoirs 16, 150 may be standard tissue culture medium models of sufficient capacity to minimize the work of re-feeding. Inlet and outlet lines extending through sturdy, rigid rods 135, 154 via autoclavable caps 130, 151 minimize the possibility of

contamination when a fresh bottle of medium is substituted in the perfusion circuit.

The actual flow rate of perfusate through pump 24 or a given setting of potentiometer 185 is determined by both viscosity of the tissue culture medium and the pressure drop incurred by the medium as it flows through cartridge 11. The precise value of the perfusate flow rate can only be determined by direct measurement since each type of cartridge 11 has its own particular resistance to flow, and because viscosity is primarily affected by serum concentration. Knowledge of a precise value is not usually necessary however. A setting of 100% on the "relative flow" dial of potentiometer 185 yields, in one embodiment, a flow rate of approximately 1300 milliliters per minute whereas in the same embodiment a 20% setting yields a flow rate of approximately 150 milliliters per minute. The latter flow rate provides sufficient oxygen in that embodiment to support about 1010 hybridoma cells. Duration of flow in any direction is set independently of the duration of flow in an opposite direction, by adjustment of potentiometers 183, 184. In some applications, it may prove advantageous to choose unequal values cf forward and reversed flow durations and thereby decrease the degree of degasing of perfusate while retaining the advantages of reversible flow.

While the compactness of reactor 10 enables it to be easily placed, along with one or more other reactors inside an incubator, the solid-state electronics controller 80 for each reactor will be positioned outside of the incubator, communicating with the pump motor inside the incubator via multi-lead ribbon cable 72. Culture medium flow rate and

periodicity of flow reversal are variables chosen by the operator for particular applications. To activate pump motor 30, switch 182 must be toggled to its "start/reset" position, and then allowed to return automatically to its "run" position.

Tissue cells may be inoculated through side ports 15a, 15b into extracapillary space of cartridge 11. Perfusate is pumped through the lumen of each fiber 12 from which nutrients diffuse through the permeable wall of the fiber into cells sitting atop the fibers; cellular waste products are removed by back diffusion through the permeable walls and into the lumina of the fibers. The direction of flow with the lumina of the fibers reverses at pre-selected intervals to enhance transport of nutrients and oxygen, to increase the effective length of the fiber bundle, and to decrease the likelihood of occlusion of the ends of the fibers by any circulating denatured serum proteins. Cartridge 11 may be inserted into the perfusion circuit via quick disconnecting couplers 20, 22, thereby enabling easy insertion of a non-autoclavable cartridge as well as transfer of a cartridge from auxiliary reactor 10 after start-up into a larger, multi-cartridge system.

Selection and use of a reactor cartridge may be a compromise between achieving maximum overall product yield and obtaining highly concentrated cell products. The choice of cartridge types depends on the type of hollow fibers best suited to attain a desired goal. Fibers differ in their abilities to support the growth of various types of cells and to concentrate specific cell products. Characteristics of importance include fiber materials, permeability, surface charge and method of sterilization. Table 2 lists

characteristics of one polypropylene cartridge type (type "A") and three DEAE--cellulose type cartridges (type "B15", "B4" and "B3").

As indicated in Table 2, polypropyrene fibers of the type "A" cartridge are steam-autoclavable, constructed of a polyolfin, and have pore sizes of about 0.5 microns. The large pore sizes enhance nutrient transport from the perfusate into cellular compartments, while decreased volumes of nutrient medium are required for hollow fiber cartridges relative to other types of cartridges, to produce a semi-concentrated product which collects within a product tank. The polypropylene cartridges must be pre-washed with dilute acid before use.

The type "B" DEAE-cellulose cartridge fibers are cellulosic and have DEAE groups bonded to their exterior extracapillary surfaces. Three types of DEAE-cellulose cartridges are available, having nominal sieving coefficients of either 3,000, 4,000 or 15,000 Daltons.

Figure 14 is a molecular weight retention chart showing the molecular weight as a function of sieving coefficient for type "B" DEAE-cellulose cartridge types. The wall of the DEAE-cellulose, non-autoclavable 4,000 Dalton fiber cartridges is thinner than that of the 4,000 Dalton autoclavable fiber. Thinner fiber walls provide increased oxygen and may permit some cell lines to grow better. A more permeable 15,000 Dalton autoclavable fiber permits certain macromolecular growth factors to diffuse from the perfusion medium into the cell compartment and thus may prove to be more effective for the growth of certain cells.

Prior to cell inoculation, power switch 180 should be placed in the "off" position. Ribbon cable 72 should be disconnected from the plug-jack pair 70 and reactor 10 placed in a laminar flow hood. Reservoir bottle 16 is then replaced with another container (e.g., a 500 milliliter bottle containing 100 milliliters of filtered, warmed, conditioned nutrient media plus an additional 100 milliliters of warm, fresh media). Tubing at both inlet and outlet perfusion ports 13a, 13b are closed using pinch clamps to prevent: backflow into the extracapillary space, the outer surfaces of side ports 15a, 15b are wiped with 70% ethanol and allowed to air dry, and 50 milliliters of complete medium containing 108 cells are drawn into a sterile sixty milliliter syringe.

Then, cartridge 11 is raised to a height above the liquid level in reservoir 16 while one side port 15a, 15b is opened and a tubing clamp is removed from the perfusion circuit at the opposite end portion 13b, 13a, respectively, allowing medium in the extracapillary space to filter back into reservoir 16. Cell suspension held by the sixty milliliter syringe may then be slowly injected into the extracapillary space via the opened side port. Upon completion of the injection, cartridge 11 is lowered to a point below the level of liquid in reservoir bottle 16 until the extracapillary space is refilled. Then the unclamped end portion 13a, 13b is again clamped and the opened side port closed. Cartridge 11 is placed in a vertical position for about one minute and then inverted during an additional minute to assure distribution of cells uniformly throughout the fiber bundle. Cartridge 11 is then placed in clips 44, both tubing clamps removed from silicone tubing coupled to end portions 13a, I3b,

cable 72 is reconnected to plug-jack pair 70, and the reactor is placed into a carbon dioxide incubator with cable 72 trailing through the door gasket, while the reactor sits for about 12 hours. Absence of perfusion circulation immediately after cell inoculation will enhance both cell attachment and conditioning of the peri-cellular micro environment.

To initiate perfusion, power switch 180 is set to its "on" position and potentiometer 185 set to a relative flow of about 10%. Potentiometers 183, 184 are both set to about one minute duty cycles and switch 181 is set to its "auto" mid-position. Then, switch 182 is toggled to its "start/reset" position to initiate perfusate circulation by pump 24. After observing small air bubbles in t:he perfusion circuit and completion of one complete flow reversal cycle, potentiometers 183, 184 may be set to longer duty cycles of about five minutes in each direction. If, after completion of several duty cycles an excessive amount of gas bubbles accumulate at one end of cartridge 11, then the length of the duty cycle which uses the adjacent end portion 13a, 13b as an inlet perfusion port may be increased by one minute while the duty cycle that uses the opposite end portion 13b, 13a as an inlet perfusion port may be increased by one minute. These adjustments should be repeated if bubbles continue to accumulate. Additionally, cartridge may be held in a vertical position to allow accumulated bubbles to return to reservoir 16. This type of degasing is unlikely to occur if either low serum containing medium or serum-free medium is used. Very high serum concentrations increase the viscosity of tissue culture medium, thereby increasing the possibility of cavitation at the pump inlet and

subsequent bubble formation at the inlet side of cartridge 11. A flow rate of about 150 milliliters per minute is appropriate for cultures during the first two weeks of inoculation.

Approximately 24-hours after initiating perfusion, reservoir 16 should be replaced with a fresh container of 100 milliliters of filtered, conditioned nutrient media and 100 milliliters of fresh nutrient medium. Perfusion with this mixture should proceed for an additional 24-hours and subsequently, the nutrient medium should be replaced every two or three days during the subsequent twelve days using one liter of fresh medium for each change. Thereafter, frequency of re-feeding will depend upon the speed with which nutrients are depleted from the medium. Each cell type is different. Some cells will produce significant quantities of acid indicating a state of nutrient depletion by a decrease in medium pH, whereas other cells will deplete glucose of glutamine without marked change in pH. If a one liter volume of nutrient medium is replaced daily however, nutrient depletion is not likely to occur. When the extracapillary space is twenty percent filled with cells, as usually occurs within two weeks after inoculation, one liter of nutrient medium should be replaced on a daily basis.

When the size of the desired cell product is smaller than the molecular weight cut-off of the capillary fibers, as is always the case with the polypropylene fibers, cell products will accumulate in reservoir 16 from which the products may then be harvested.

If cell products are larger than the molecular weight cut-off, as is usually the situation when using the DEAE-cellulose type cartridges, cell

products will accumulate in the extracapillary space and can be harvested. In preparation for harvesting cell products from the extracapillary space of DEAE-cellulose cartridges, perfusion circuit tubing at both end sections 13a, 13b of the cartridge must be closed using tubing clamps. Side ports 15a, 15b must be sterilized as by wiping with 70% of ethanol and allowed to air dry in a laminar flow hood. Cartridge 11 must then be raised to the level of, or slightly above, the level of liquid nutrient medium in reservoir 16 and side ports 15a, 15b opened. Cartridge 11 may then be tilted to permit aspiration of extracapillary contents with a sixty milliliter syringe fitted with a long, extra bore sterile needle. Upon completion, the extracapillary space should be refilled with a like amount of complete nutrient medium using a fresh syringe, side ports 15a, 15b closed, the tubing clamps removed, perfusate circulation re-started and reactor 10 replaced into an incubator.

It may be noted that some cell types grow or function substantially better in vitro when they have adhered or attached to a solid or semi-solid substance. These cells are frequently termed "anchorage", or "attachment-dependent" cell types.

The polyolifinic hollow fibers do not lend themselves well to cell attachment, i.e., while they support non-anchorage dependent cells well in the hollow fiber bioreactor, they do not provide an adequate growth matrix for anchorage-dependent cells. For this reason, it is, on occasion, of use to modify the surface of such fibers with a material that enables cells to adhere to their surface. For example, a bioadhesive obtained from barnacles and marketed by Biopolymers, Inc. under the trademark Celltak can be

used to coat hollow fibers and improve cell attachment. Other materials exist and can be for example, various collagen types.

In addition, cells can be grown on microcarriers, minute particles of non-cytotoxic materials, for example, dextran beads. Inoculum has been mixed with one gram of pre-swelled, sterile CytodexTM (from Pharmacia) beads and then inoculated this into the loading side ports of the bioreactors. The cells attach to the beads. The beads lodge between the fibers or rest atop the fibers, close enough that their adherent cells can be fed by the supporting hollow fibers. A 3-fold improvement in cell secretion using this approach with one cell line has been observed.

It is evident that those skilled in the art may now make numerous uses and modifications (for example, by substituting others of the numerous autoclavable, Food and Drug Administration approved materials for some of the materials disclosed herein) of any departures from the specific embodiments described herein without departing from the inventive concepts disclosed. For example, the timer stages shown in Figure 12 endow the embodiment with a measure of inherent reliability through their self-resetting configuration. Their timing function may however, be performed by a microprocessor. Although specific examples of containers such as standard tissue culture bottles are shown in the several figures for use as reservoirs 16, 150, and a specific tube for use as reservoir 160, these examples are only suggestive of the wide diversity of containers with which the invention may be practiced. Moreover, although the specific supporting structure including tray 40,

handles 42, clips 44 and racks 48 disclosed advantageously enables the entire reactor to be placed within both standard, commercially available, laboratory-size incubators and autoclaves, the shape and design of the supporting structure serve principally to illustrate the principles of the invention and may be modified without departing from those principles.

Consequently, the invention is to be construed as embracing each end every novel feature and combination of features present in or possessed by the apparatus and process herein disclosed, and is to be limited solely by the spirit and scope of the appended claims.

CLAIMS

1. An in vitro cell culture reactor, comprising:

first reservoir means for storing a nutrient medium;

gas exchanging means including a first end and a second end, connectable in a perfusion circuit to said first reservoir for passing perfusate containing said nutrient medium between said first and second ends while exposing the perfusate to oxygen and carbon dioxide gases ambient to said gas exchanging means;

a cell culture cartridge including:

a shell connectable into said perfusion circuit via spaced end portions and defining an elongated chamber there between, one of said end portions being connectable to one of said first and second ends of said gas exchanging means; and

capillary means for simulating a vascular network within said chamber, said capillary means including a multiplicity of individual capillaries extending said perfusion circuit between said spaced end portions, some of said capillaries having walls which are permeable to nutrients of said nutrient medium and/or cell products, said capillaries dividing said chamber into intracapillary space within said capillaries and extracapillary space outside of said capillaries, said intracapillary space and extracapillary space communicating with each other only through the walls of said capillaries, said capillaries being spaced from each other to provide said

extracapillary space for three-dimensional growth of cells and to enable cells growing on and between capillaries to obtain nourishment from perfusate passing through said capillaries and to discharge waste products via perfusate passing through said capillaries; pumping means connectable between said reservoir and the other of said end portions for circulating perfusate from said first reservoir through said perfusion circuit in a forward direction during a first phase and in a reversed direction during a second phase; and

controlling means for periodically enhancing transport of nutrients and gases through said intracapillary space by controlling operation of said pumping means to provide independently variable duty cycles for both said first and second phases.

2. The reactor of claim 1, further comprised of said controlling means controlling said pumping means to circulate perfusate from said first reservoir, through one of said end portions, through said intracapillary space, through said gas exchanging means, and into said reservoir during said first phase, and to circulate perfusate from said reservoir, through said gas exchanging means, through the other of said end portions, through said intracapillary space and into said reservoir during said second phase.

3. The reactor of claim 1, wherein said first reservoir means comprises:

a first container including an uppermost portion providing a sole opening into said first container; and

a first coupler forming a sealing relation between environment ambient to said first container and said sole opening, said first coupler including a first port connectable to said first side of said pump, a second port connectable to the other of said first end and second end of said gas exchanging means, and a substantially rigid elongated element extending vertically downward into said first container via said sole opening with a first lumen communicating between one of said first and second ports of said first coupler and the interior of said first container, and a second and longer lumen extending vertically downward beyond said first lumen communicating between the other one of said first and second ports and the interior of said first container.

4. The reactor of claim 1, wherein: said gas exchanging means comprises a length of tubing of a non-cytotoxic material permeable to oxygen and carbon dioxide gases, extending between said first and second ends; and supporting means including a base and first and second spaced apart, vertically extending racks engaging said base while supporting said length of tubing in a serially wound coil surrounding said pumping means and said first reservoir, for retaining said length of tubing, cell culture cartridge, pumping means and first reservoir in a connected configuration of said perfusion circuit.

5. The reactor of claim 4, wherein said supporting means firmly and removeably engages said cell culture cartridge while exposing said elongated chamber to direct visual observation.

6. The reactor of claim 4, wherein said supporting means further comprises a plurasitc of clips mounted in an aligned array along one side of said base

and biased removeable, friction-fit engagement with said shell being exposed to direct visual observation.

7. The reactor of claim 4, wherein said supporting means further comprises a plurality of clips mounted in an aligned array spaced apart from said racks along one side of said base, and biased to receive and hold said cartridge in a removeable, friction-fit engagement with said shell being spaced apart alongside said coil and being exposed to direct visual observation.

8. The reactor of claim 4, wherein: said racks are each perforated by a plurality of holes exposing adjacent exterior surfaces of said tubing to the oxygen and carbon dioxide gases ambient to said tubing; and

a plurality of tabs extend outwardly from a first plurality of said holes in each of said racks to form spaced apart surfaces supportingly engaging successive segments of windings of said serially wound coil.

9. The reactor of claim 1, further comprising: a first coupling within said perfusate circuit connecting said one end portion with one of said first and second ends; and

second coupling within said perfusate circuit connecting said other end portion with said pumping means;

both of said first and second couplings having interchangeable female element:s: and male elements, each male element removeably receiving a female element to complete said perfusate circuit;

each of said end portions being fitted with interchangeable ones of said female and male elements.

10. The reactor of claim 9, further comprising: a base;

first and second spaced apart racks engaging and extending transversely from a major surface of said base while supporting said gas exchanging means in an ordered configuration with substantially full exposure to environment ambient to said supporting means; and

a plurality of clips mounted in an aligned array spaced apart from said racks along one side of said base, and biased to receive and hold said cartridge in a removeable, friction-fit engagement with said shell being spaced apart alongside said gas exchanging means and being exposed to direct visual observation.

11. The reactor of claim 1, wherein:

said cell culture cartridge includes:

first means for communicating with said extracapillary space; and

second means spaced apart from said first communicating means by a major length of said shell, for communicating with said extracapillary space;

said reactor further comprising: second reservoir means connectable to said extracapillary space via one of said first and second communicating means, for storing and introducing cell suspension and/or cell culture medium into said extracapillary space via said one of said first and second communicating means; and

orifice means coupled to said second reservoir means, for subjecting cell culture within said second reservoir means to a pressure urging the cell suspension and/or cell culture medium into said extracapillary space; and

third reservoir means connectable to said extracapillary space via the other of said first and second communicating means, for receiving matter from said extracapillary space under influence of said pressure.

12. The reactor of claim 11, wherein: said second reservoir means comprises a first container including an uppermost portion providing a top opening into said first container; and

said orifice means includes a first coupler forming a sealing relation between environment ambient to said first container and said top opening, a first orifice connectable to one of said first and second communicating means, a second orifice connectable to a source of said pressure, and a substantially rigid elongated element extending vertically downward into said first container via said top opening with a conduit communicating between said first orifice of said orifice means and the interior of said first container independently of said second orifice.

13. The reactor of claim 3, wherein: said cell culture cartridge includes:

first means for communicating with said extracapillary space; and

second means spaced apart from said first communicating means by a major length of said shell, for communicating with said extracapillary space; said reactor further comprising:

second reservoir means connectable to said tracapillary space via one of said first and second mmunicating means, for storing and introducing cell culture into said extracapillary space via said one of its first and second communicating means; and

orifice means coupled to said second reservoir means, for subjecting cell culture within said second reservoir means to a pressure during the cell culture to said extracapillary space; and

third reservoir means connectable to said extracapillary space via the other of said first and second communicating means, for receiving matter from said extracapillary space under influence of said pressure.

14. The reactor of claim 13, wherein: said second reservoir comprises a second container including an uppermost portion providing a top opening into said second container, and

said orifice means includes a second coupler forming a sealing relation between environment ambient to said second container and said top opening, a first orifice connectable to one of said first and second communicating means, a second orifice connectable to a source of said pressure; and a substantially rigid elongated element extending vertically downward into second container via said upper opening with a conduit communicating between said first orifice and the said second container independently of said orifice.

15. The reactor of claim 1, wherein said pumping
means comprises:

driven means providing a sealed unit accessible only via inlet and outlet ports, coupled via said inlet and outlet ports into said perfusate circuit and having a moving member disposed wholly within said sealed unit between said inlet and outlet ports while immersed for urging perfusate flowing through said perfusate circuit, for uring perfusate to flow

between said inlet and outlet ports;

driving means including a disengageable coupler forming a couplement with and controlling movement of said moving member, for driving said moving member by imparting motion to said disengageable coupler;

housing means for mounting said driven means in a fixed position relative to said driving means while allowing one degree of freedom of movement by said moving member, and for removeably holding said driving means in a friction-fit engagement with said disengageable coupler engaging said moving member. while permitting complete disengagement of said driven and driving means simultaneously with unidirectional removal of said driven means from said housing means restrained only by forces due to gravity, said couplement and said friction-fit engagement; and

detent means for restraining relative rotation between said driving means and housing while permitting free movement of said disengageable coupler under influence of said driving means.

16. The reactor of claim 15, wherein one of said moving means and said disengageable coupler include a magnet while the other of said moving means and said disengageable coupler include a ferromagnetic element disposed to form a magnetic circuit with and to freely move under influence of said magnet.

17. The reactor of claim 15, wherein: said moving means comprises a magnetically responsive element disposed to freely move said moving member relative to perfusate within said perfusate circuit; and

said disengageable coupler includes a magnet magnetically engaging and forming a magnetic circuit with said magnetically responsive element.

18. The reactor of claim 10, wherein said pumping means comprises:

driven means providing a sealed unit accessible only via inlet and outlet ports, coupled via said inlet and outlet ports into said perfusate circuit and having a moving member disposed wholly within said sealed unit between said inlet and outlet ports while immersed within perfusate flowing through said perfusate circuit, for urging perfusate to flow between said inlet and outlet ports;

driving means including a disengageable coupler forming a magnetic couplement with and controlling movement of said moving member, for driving said moving member by imparting motion to said disengageable coupler;

housing means for mounting said driven means in a fixed position relative to said base while allowing one degree of freedom of movement by said moving member, and for removeably holding said driving means in a friction-fit engagement with said disengageable coupler engaging said moving member, while permitting complete disengagement of said driven and driving means simultaneously with unidirectional removal of said driven means from said housing means restrained only by forces due to gravity, said magnetic couplement and said friction-fit engagement; and

detent means for restraining relative rotation between said driving means and housing while permitting free movement of said disengageable coupler under influence of said driving means.

19. The reactor of claim 1, wherein said capillary means comprises steam-autoclavable hollow fibers of a polyolfin having a pore size on the order 0.5 microns.

20. The reactor of claim 1, wherein said capillary means comprises hollow fibers of a cellulosic having DEAE groups bonded to their cellulosic moeity.

21. The reactor of claim 1, wherein said controlling means comprises:

first timer means including a first adjustment stage, for responding to a first logic level of a reset signal by generating a first output signal exhibiting a first logic state during a duty cycle determined by said first adjustment stage to define said first phase, and by responding to a second logic level of a reset signal by generating said first output signal exhibiting a second logic state;

second timer means including a second adjustment stage distinct from said first adjustment stage, for responding to said first output signal exhibiting said first logic state by generating and applying to said first timer means a reset signal exhibiting said first logic level, and for responding to said first output signal exhibiting said second logic state by generating a reset signal exhibiting said second logic level during a duty cycle determined by said second adjustment stage to define said second phase; and

multiplexing means for regulating said pumping means to circulate said perfusate in said forward direction upon reception of said first output signal exhibiting said first logic state, and to circulate said perfusate in said reversed direction upon reception of said first output signal exhibiting

said second logic level.

22. The reactor of claim 21, wherein said controlling means is further comprised of latching means for governing said pumping means to circulate the perfusate in response to reception at a first terminal of a reset signal exhibiting said first logic level, and for interrupting circulation of the perfusate by said pumping means during reception at a second terminal of a reset signal exhibiting said first logic level.

23. The reactor of claim 22, wherein said controlling means further comprises first switching means providing a first switched position, for temporarily and simultaneously applying a reset signal exhibiting said first logic level to said first timer means and said first terminal of said latching means, providing a second switched position for continuously interrupting application of any reset signal to said latching means, and providing a third switched position for applying a reset signal exhibiting said first logic level to said second terminal of said latching means.

24. The reactor of claim 1, wherein said controlling means comprises:

first timer means including a first adjustment stage, for responding to a first logic level of a reset signal by generating a first output signal exhibiting a first logic state during a duty cycle determined by said first adjustment stage to define said first phase, and by responding to a second logic level of a reset signal by generating said first output signal exhibiting a second logic state;

second timer means including a second adjustment stage distinct from said first adjustment stage, for responding to said first output signal

exhibiting said first logic state by generating and applying to said first timer means a reset signal exhibiting said first logic level, and for responding to said first output signal exhibiting said second logic state during said second phase having a duty cycle determined by said second adjustment stage by generating a reset signal exhibiting said second logic level during a duty cycle determined by said second adjustment stage to define said second phase; and

multiplexing means for regulating said pumping means to circulate said perfusate in said forward direction upon reception at a first electrode of said first output signal exhibiting said first logic state, to circulate said perfusate in said forward direction upon reception at a second electrode of a first input signal exhibiting said first logic state, to circulate said perfusate in said reversed direction upon reception at said first electrode of said first output signal exhibiting said second logic level, and to circulate said perfusate in said reversed direction upon reception at a third electrode of said first input signal exhibiting said first logic state.

25. The reactor of claim 24, wherein said controlling means is further comprised of latching means for governing said pumping means to circulate the perfusate in response to reception at a first terminal of a reset signal exhibiting said first logic level, and for interrupting circulation of the perfusate by said pumping means during reception at a second terminal of a reset signal exhibiting said first logic level.

26. The reactor of claim 25, wherein said controlling means further comprises first switching means providing a first switched position, for

temporarily and simultaneously applying a reset signal exhibiting said first logic level to said first timer means and said first terminal of said latching means, providing a second switched position for continuously interrupting application of any reset signal to said latching means, and providing a third switched position for applying a reset signal exhibiting said first logic level to said second terminal of said latching means.

27. An in vitro cell culture reactor, comprising:

first reservoir means for storing a nutrient medium;

a length of hollow tubing of a non-cytotoxic material permeable to oxygen and carbon dioxide gases:, having open first and second ends connectable in a perfusion circuit directly to said first reservoir, for passing perfusate containing a liquid nutrient media between said first and second ends while exposing the perfusate to gases ambient to said tubing; a cell culture cartridge including:

a shell connectable into said perfusion circuit via spaced end portions and defining an elongated chamber there between, one of said end portions being connectable directly to one of said upper and lower ends of said tubing; and

capillary means for simulating a vascular network within said chamber, said capillary means including a multiplicity of individual capillaries extending said perfusion circuit between said spaced end portions, said capillaries having walls which are permeable to nutrients of a liquid nutrient media and to cell products, said capillaries dividing said chamber into intracapillary space within said capillaries and extracapillary space outside of said

capillaries, said intracapillary space and extracapillary space communicating with each other only through the walls of said capillaries, said capillaries being spaced from each other to provide said extracapillary space for three-dimensional growth of cells and to enable cells growing on and between capillaries to obtain nourishment from perfusate passing through said capillaries and to discharge waste products via perfusate passing through said capillaries;

pumping means connectable between said reservoir and the other of said end portions for circulating perfusate from said first reservoir through said perfusion circuit in a forward direction during a first phase and in a reversed direction during a second phase;

controlling means for periodically enhancing transport of nutrients and gases through said intracapillary space by controlling operation of said pumping means to provide independently variable duty cycles for both said first and second phases; and

supporting means providing a portable, hand-holdable rigid base and first and second spaced apart racks engaging and transversely extending from said base while supporting said tubing in a serially wound helical coil surrounding said pumping means and said first reservoir, said base retaining said cell culture unit, pumping means and first reservoir in an ordered alterably connected configuration of said perfusion circuit;

said racks being each perforated by a plurality of holes exposing adjacent exterior surfaces of said tubing to the oxygen and carbon dioxide gases ambient to said tubing; and

a plurality of tabs extend outwardly from a first plurality of said holes in each of said racks to form spaced apart surfaces supportingly engaging successive segments of windings of said seriallY wound coil.

28. The reactor of claim 27, further comprised of said controlling means controlling said pumping means to circulate perfusate from said first reservoir, through one of said end portions through said intracapillary space, through said tubing, and into said reservoir during said first phase, and to circulate perfusate from said reservoir, through said tubing, through the other of said end portions, through said intracapillary space and into said reservoir during said second phase.

29. An auxiliary in vitro cell culture reactor, comprising:

first reservoir means for storing a nutrient medium;

stationary gas exchanging means including an upper end and a lower end, connectable in a perfusion circuit to said first reservoir for passing perfusate containing said nutrient medium between said upper and lower ends while exposing the perfusate to oxygen and carbon dioxide gases ambient to said gas exchanging means via said first coupling;

a first coupling within said perfusate circuit and connected to one of said upper and lower ends;

a cell culture cartridge including:

a shell connectable into said perfusion circuit via spaced end portions and defining an elongated chamber there between, one of said end portions being connectable to one of said upper and

lower ends of said gas exchanging means;

capillary means for simulating a vascular network within said chamber, said capillary means including a multiplicity of individual capillaries extending said perfusion circuit between said spaced end portions, said capillaries having walls which are permeable to nutrients of said nutrient medium, to cell products and to gases, said capillaries dividing said chamber into intracapillary space within said capillaries and extracapillary space outside of said capillaries, said intracapillary space and extracapillary space communicating with each other only through the walls of said capillaries, said capillaries being spaced from each other to provide said extracapillary space for three-dimensional growth of cells and to enable cells growing on and between capillaries to obtain nourishment from perfusate passing through said capillaries and to discharge waste products via perfusate passing through said capillaries;

first means for communicating with said extracapillary space; and

second means spaced apart from said first communicating means by a major lengt of said shell, for communicating with said extracapillary space;

a second coupling within said perfusate circuit and connected to the other end portion;

pumping means connectable between said other end portion via said second coupler and said reservoir, for circulating perfusate from said first reservoir through said perfusion circuit in a forward direction during a first phase and in a reversed direction during a second phase;

controlling means for periodically enhancing transport of nutrients and gases through said intracapillary space by controlling operation of said pumping means to provide independently variable duty cycles for both Said first and second phases; and

supporting means providing a portable, hand-holdable rigid base and a plurality of clips mounted in an aligned array spaced apart from said gas exchanging means along one side of said base, and biased to receive and hold said cartridge in a removeable, friction-fit engagement with said shell being spaced apart alongside said gas exchanging means and being exposed to direct visual observation, retaining said gas exchanging means, cell culture unit, pumping means and first reservoir in an ordered alterably connected configuration of said perfusion circuit;

both of said first and second couplings having interchangeable female elements and male elements, each male element removeably receiving a female element to complete said perfusate circuit;

each of said end portions being fitted with interchangeable ones of said female and male elements.

30. The reactor of claim 29, further comprised of said controlling means controlling said pumping means to circulate perfusate from said first reservoir, through one of said end portions through said intracapillary space, through said gas exchanging means, and into said reservoir during said first phase, and to circulate perfusate from said reservoir, through said gas exchanging means, through the other of said end portions, through said intracapillary space and into said reservoir during said second phase.

31. The reactor of claim 29, wherein said first reservoir means comprises:

a first container including an uppermost portion providing a sole opening into said first container; and

a first coupler forming a sealing relation between environment ambient to said first container and said sole opening, said first coupler including a first port connectable to said first side of said pump, a second port connectable to the other of said upper end and lower end of said gas exchanging means, and a substantially rigid elongated element extending vertically downward into said first container via said sole opening with a first lumen communicating between one of said first and second ports of said first coupler and the interior of said first container, and a second and longer lumen extending vertically downward beyond said first lumen communicating between the other one of said first and second ports and the interior of said first container.

32. An auxiliary in vitro cell culture reactor, comprising:

a first container including an uppermost portion providing a sole opening directly into said first container;

a first coupler forming a sealing relation between environment ambient to said first container and said sole opening, said first coupler including a first port, second port and a rigid elongated element extending vertically downward into said first container via said sole opening with a first lumen communicating between one of said first and second ports of said first coupler and the interior of said first container, and a second and longer lumen extending vertically downward beyond said first lumen and communicating between the other one of said first and second ports

and the interior of said first container;

a length of hollow tubing of a non-cytotoxic material permeable to oxygen and carbon dioxide gases, having open upper and lower ends connectable in a perfusion circuit to said second port, for passing perfusate containing a liquid nutrient media between said upper and lower ends while exposing the perfusate to oxygen and carbon dioxide gases ambient to said tubing;

a cell culture cartridge including:

a shell connectable into said perfusion circuit via spaced end portions and defining an elongated chamber there between, one of said end portions being connectable to one of said upper and lower ends of said gas exchanging means; and

Capillary means for simulating a vascular network within said chamber, said capillary means including a multiplicity of individual capillaries extending said perfusion circuit between said spaced end portions, said capillaries having walls which are permeable to nutrients of a liquid nutrient media and to cell products , said capillaries dividing said chamber into intracapillary space within said capillaries and extracapillary space outside of said capillaries, said intracapillary space and extracapillary space communicating with each other only through the walls of said capillaries, said capillaries being spaced from each other to provide said extracapillary space for three-dimensional growth of cells and to enable cells growing on and between capillaries to

obtain nourishment from perfusate passing through said capillaries and to discharge waste products via perfusate passing through said capillaries; pumping means connectable between said first port of said reservoir and the other of said end portions for circulating perfusate from said first reservoir through said perfusion circuit in a forward direction during a first phase and in a reversed direction during a second phase; controlling means for periodically enhancing transport of nutrients and gases through said intracapillary space by controlling operation of said pumping means to provide independently variable duty cycles for both said first and second phases; and

supporting means providing a portable, hand-holdable rigid base while retaining said tubing, cell culture unit, pumping means and first container in an ordered, alterable connected configuration of said perfusion circuit.

33. The reactor of claim 32, wherein said first coupler further comprises:

a skirt structurally conforming to and removeably engaging a neck of said first container adjoining said sole opening;

a base adjoining and closing one and if said skirt; sealing means disposed within said skirt, for forming a sealing relation between environment ambient to said first container and the interior of said first container; and

a rigid elongated element in a spaced apart relation to said skirt, extending through said base and skirt, and being insertable into the interior of said first container via the sole opening, with first and

second discreet ports disposed on one side of said base opposite to said sealing means, a first lumen communicating between one of said first and second ports and the interior of said first container and a second lumen extending further into the interior of said first container than said first lumen and communicating between the other of said first and second ports and the interior of the container.

34. A container cap, comprising: a skirt structurally conforming to and removeably engaging a neck of a container adjoining an opening into an interior of the container;

a base adjoining and closing one end of said skirt; sealing means disposed within said skirt adjacent to said base, for forming a sealing relation between environment ambient to the container and the interior of the container; and

a rigid elongated element in a spaced apart relation to said skirt, extending through said base and said skirt, and insertable into the interior of the container via the opening, with first and second discreet ports disposed on one side of said base opposite to said sealing means, a first lumen communicating between one of said first and second ports and the interior of the container and a second lumen extending further into the interior of the container than said first lumen and communicating between the other of said first and second ports and the interior of the container.

35. The container cap of claim 34, further comprising a closeable third port disposed on an exterior surface of and communicating with the interior interior container via said skirt.

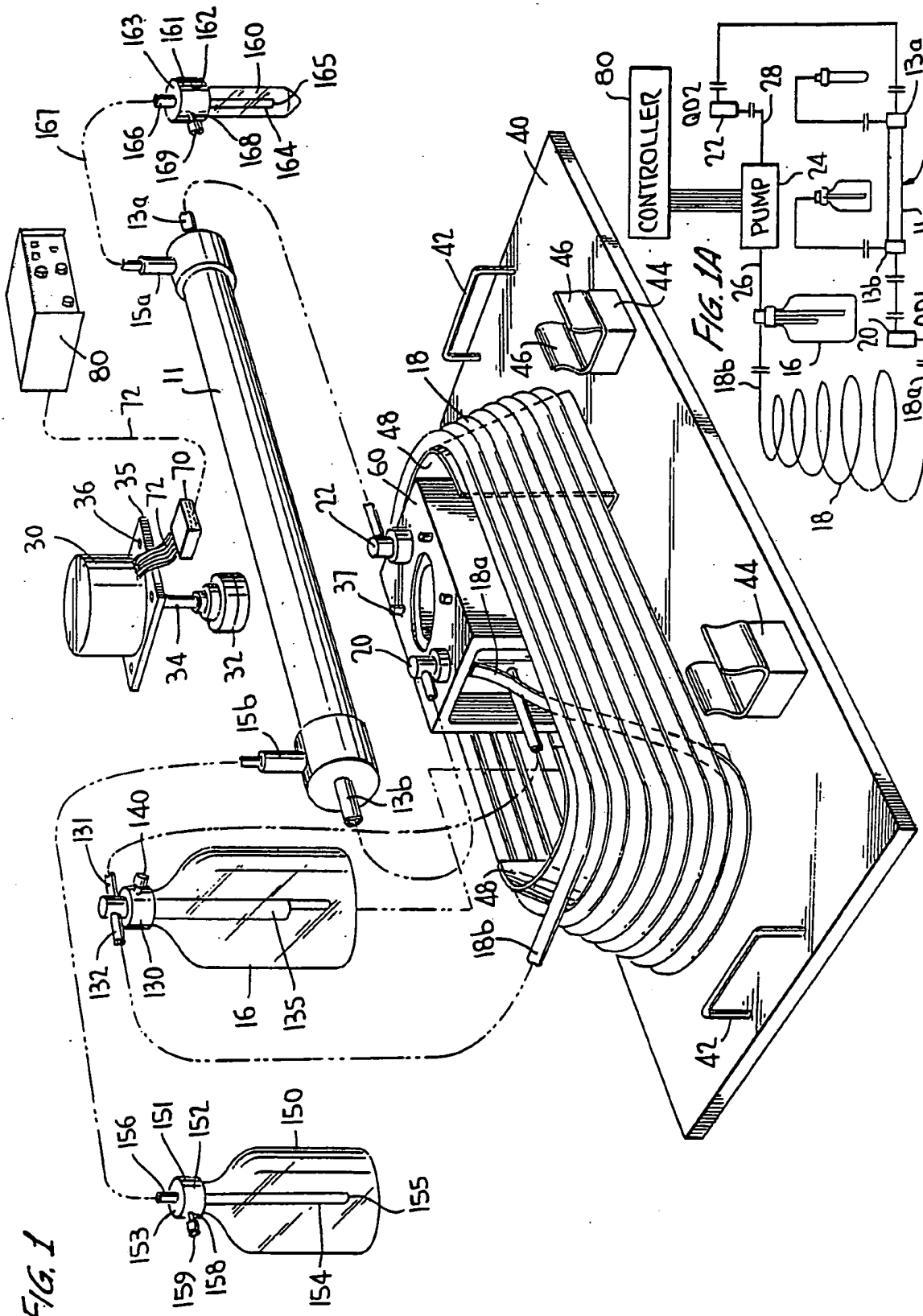

CONTROLLER
PUMP
UNIT
80
40
42
44
46
48
18
18a
18b
16
20
22
24
26
28
11
13a
13b
Q01
Q02
30
32
34
35
36
37
60
70
72
131
132
130
135
140
150
151
152
153
154
155
156
158
159
160
161
162
163
164
165
166
167
168
169

FIG. 1A

FIG. 1

FIG. 2

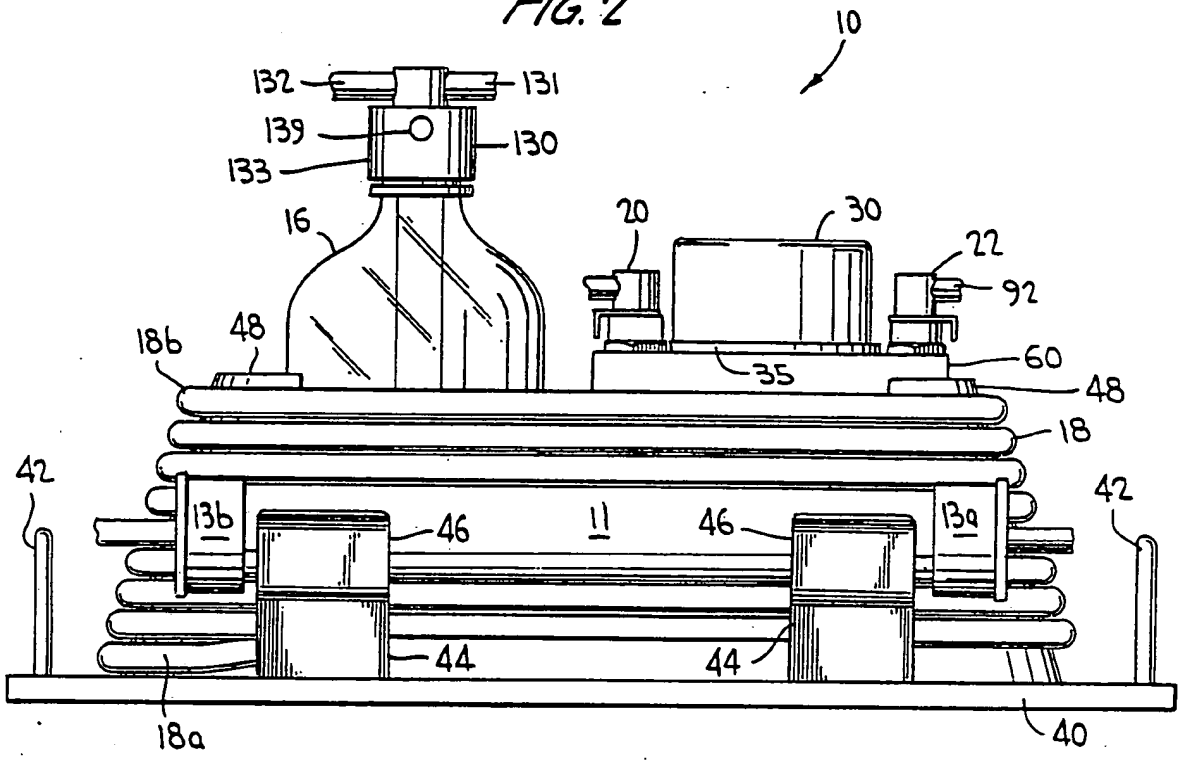

10
132
131
139
130
133
16
20
30
22
92
186
48
35
60
48
18
42
136
46
11
46
136
42
44
44
18a
40

FIG. 3

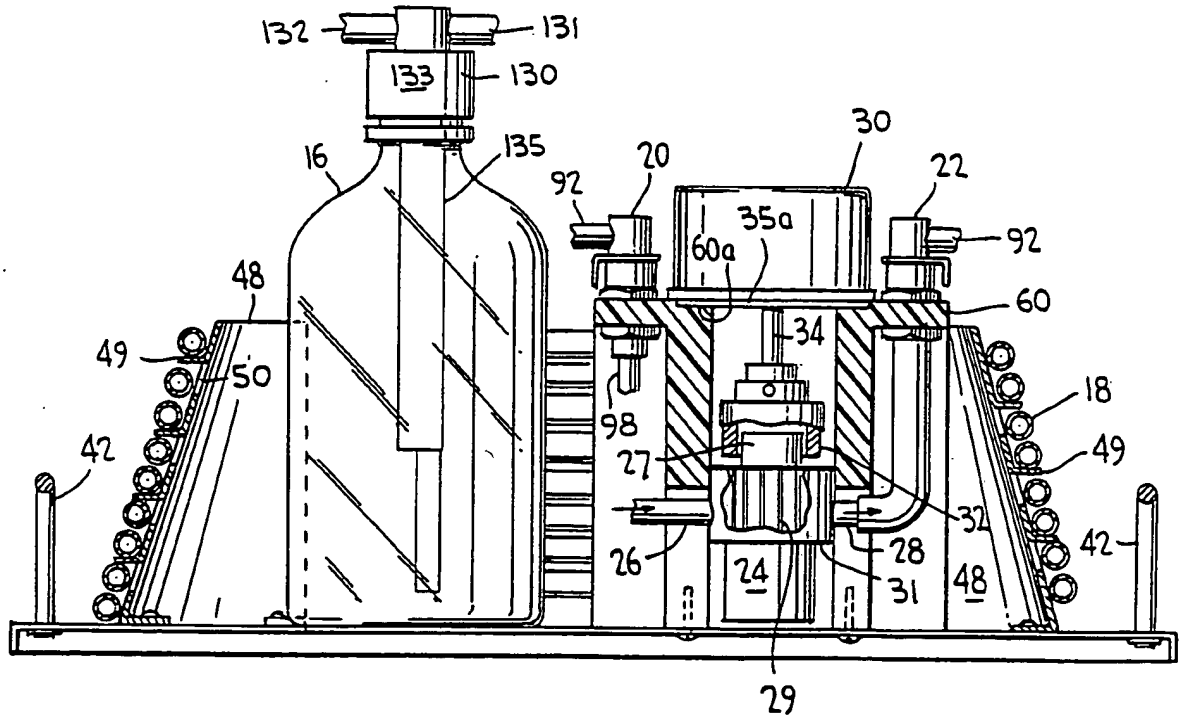

132
131
133
130
135
16
92
20
30
22
35a
60a
92
48
60
34
49
50
18
98
42
27
49
32
26
28
42
24
31
48
29

FIG. 4

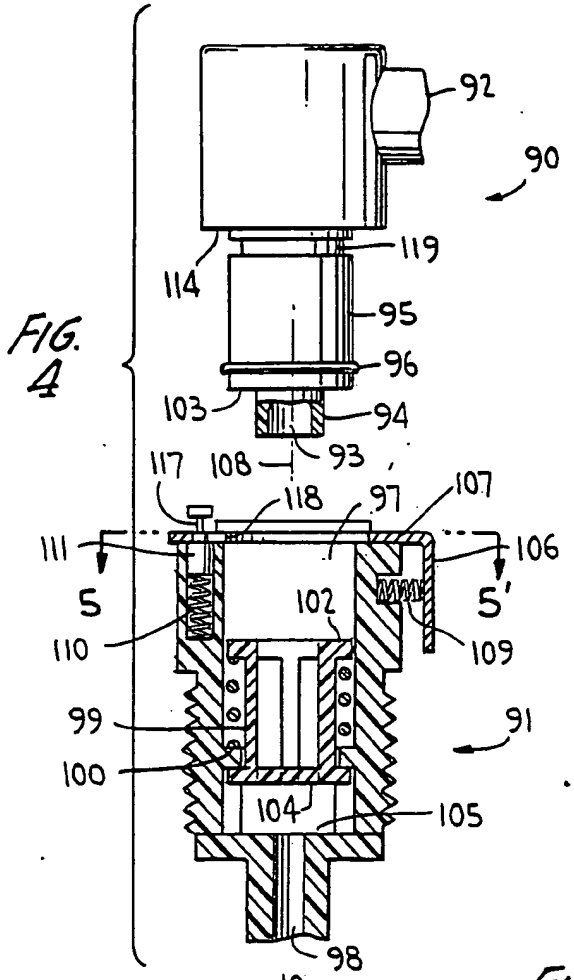


FIG. 5

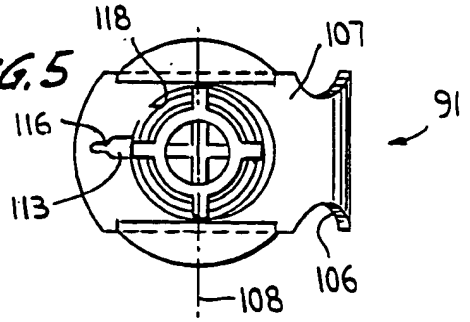


FIG. 6

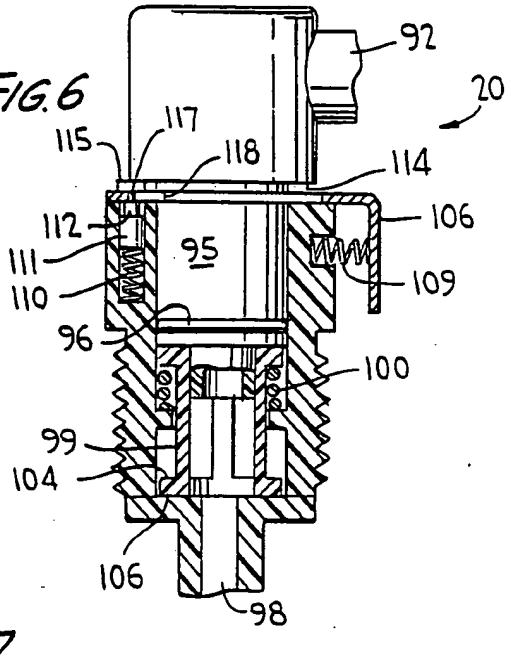


FIG. 7

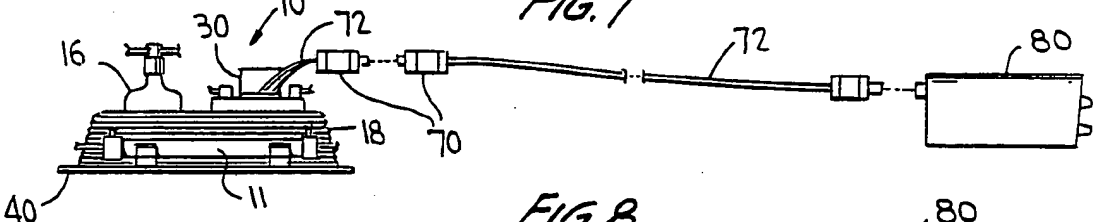
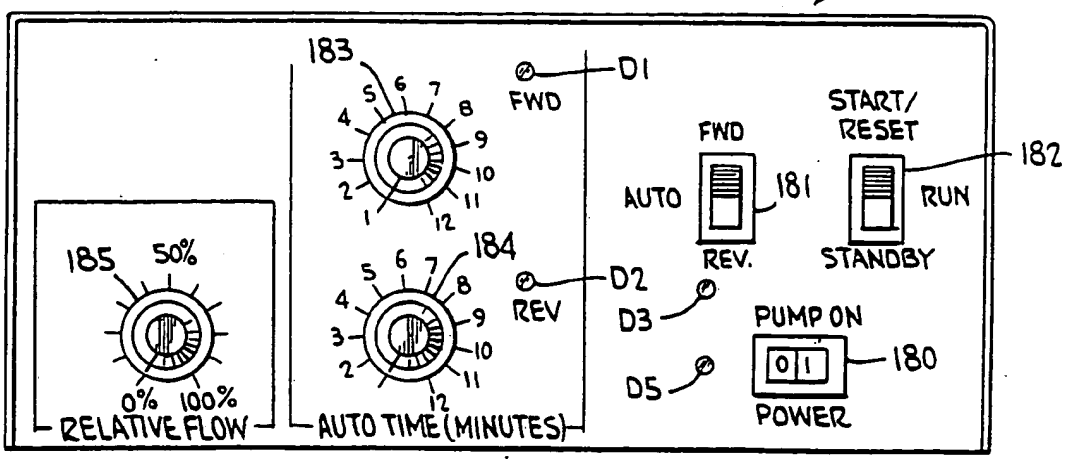


FIG. 8



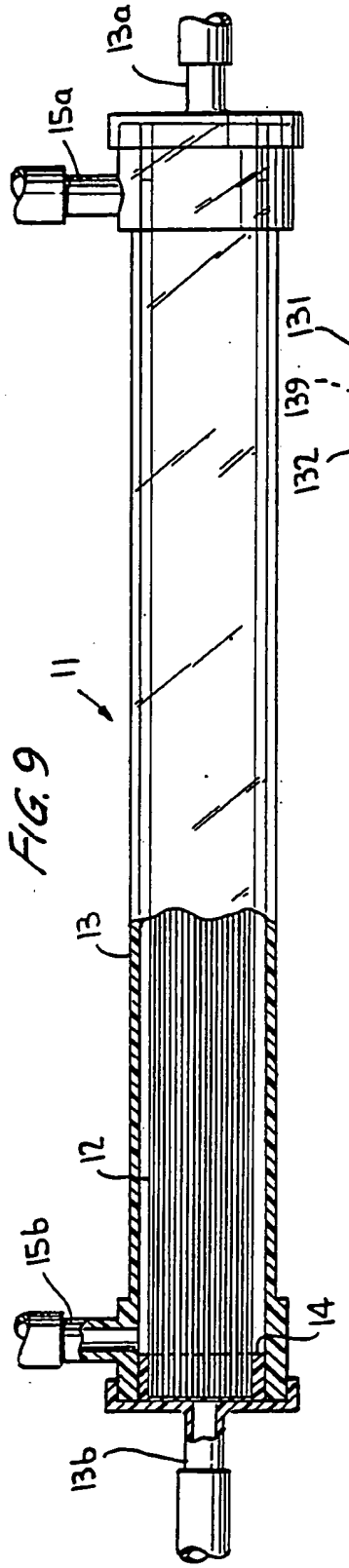


FIG. 9

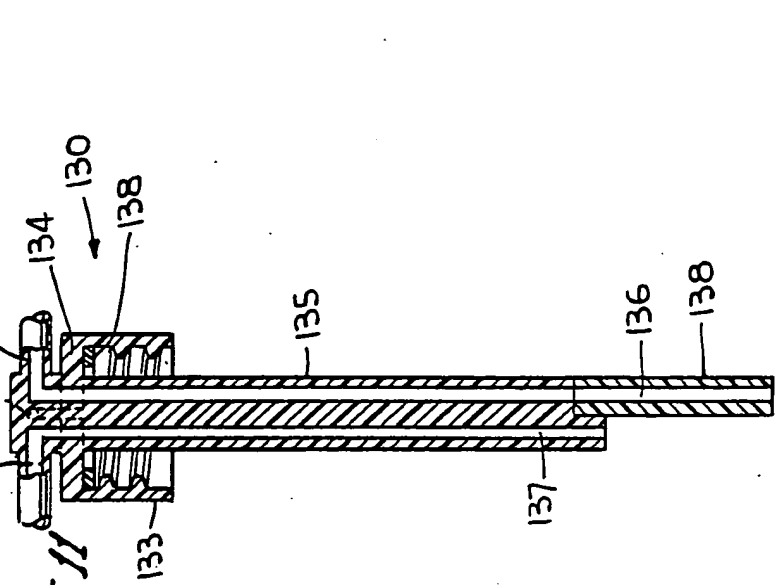
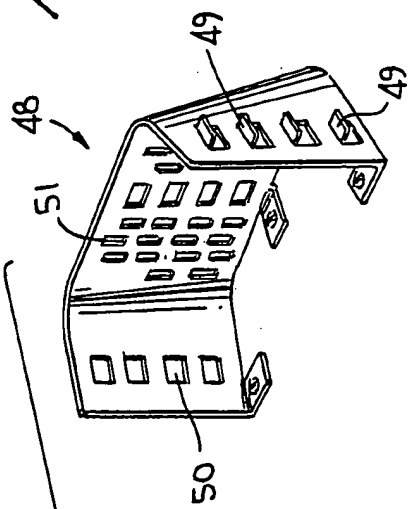


FIG. 11



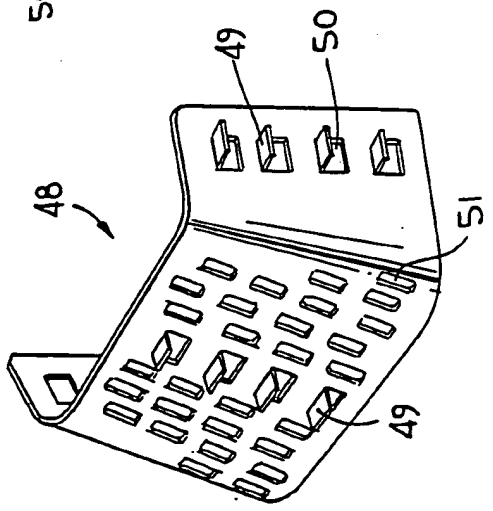
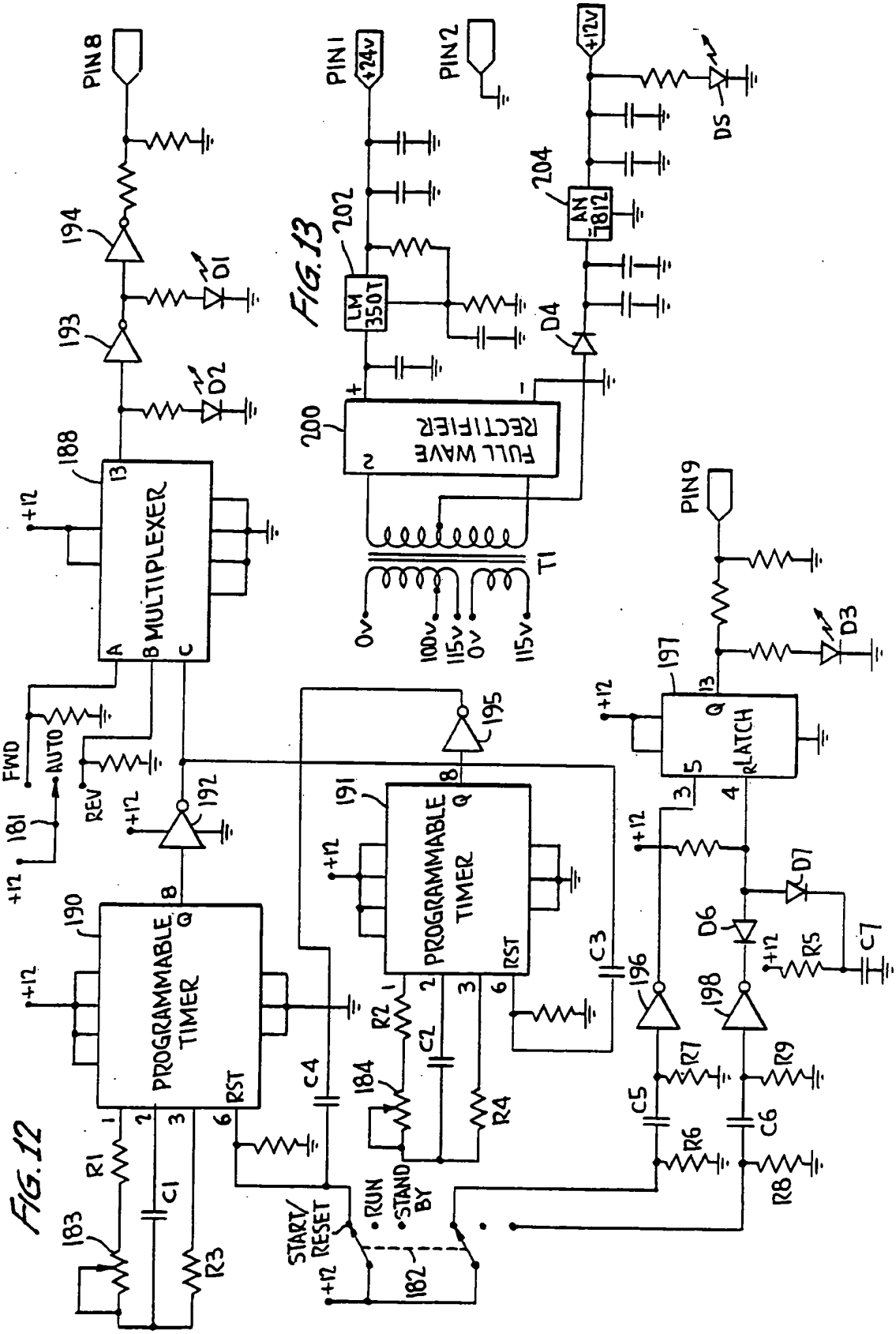


FIG. 10


PIN 8
PIN 1
+24v
PIN 2
+12v
DS
204
AN 1812
202
LM 350T
FIG. 13
D4
200
FULL WAVE RECTIFIER
T1
0v
100v
115v
0v
115v
194
D1
193
D2
188
MULTIPLEXER
A
B
C
13
+12
PIN 9
D3
197
LATCH
Q
R
FWD
AUTO
REV
181
192
195
191
PROGRAMMABLE TIMER
190
PROGRAMMABLE TIMER
RST
C3
196
198
D7
D6
R5
C7
R2
C2
R4
184
C4
R1
C1
R3
183
R7
R9
C5
C6
R6
R8
START/ RESET
RUN
STAND BY
182
FIG. 12

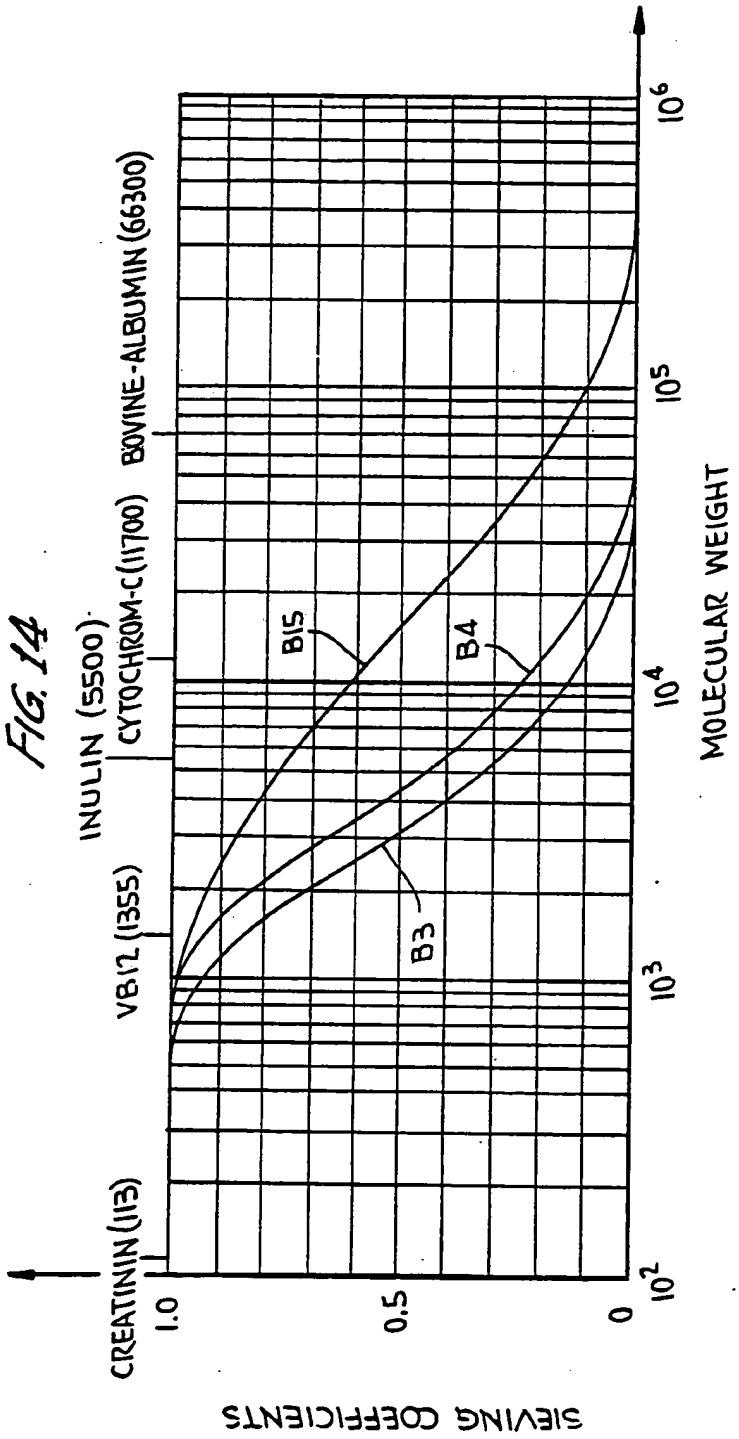

BOVINE-ALBUMIN (66300)
CYTOCHROM-C (11700)
INULIN (5500)
VB12 (1355)
CREATININ (113)
B15
B4
B3
10^6
10^5
10^4
10^3
10^2
MOLECULAR WEIGHT
1.0
0.5
0
SIEVING COEFFICIENTS

FIG. 14

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/03644

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): C12M 3/04, 1/24, 1/04

U.S. Cl. 435/285, 296, 313

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/284, 285, 286, 288, 289, 296, 311, 313, 818; 210/321.64, 321.69, 321.72, 321.78, 321.79, 321.8; 215/228, 270, 307, 309

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y,P	New Brunswick Scientific Product Bulletin, issued June 1989, (New Brunswick Scientific Co., Inc., Edison, New Jersey), "CellTronics HF-100: The Bench-Top Hollow Fiber Bioreactor System", see pages 1 to 4.	1-35
Y	JP, A, 61-88872 (SNOW BRAND MILK PROD. CO. LTD.) 07 May 1986, see entire document.	1-33
Y	US, A, 4,220,725 (KNAZEK) 02 September 1980, see column 2, line 29 to column 3, line 20 and column 3, line 55 to column 4, line 25.	1-33
Y	US, A, 3,821,087 (KNAZEK) 28 June 1974, see column 2, line 48 to column 4, line 24.	1-33
Y	US, A, 4,649,114 (MILTENBURGER) 10 March 1987, see column 3, lines 22 to 34 and column 3, line 56 to column 4, line 32.	1-33
Y	US, A, 3,893,887 (SMITH) 08 July 1975, see column 3, lines 9 to 22.	33, 34, 35

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 November 1989	29 NOV 1989

International Searching Authority	Signature of Authorized Officer
ISA/US	Randall E. Deck

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	US, A, 4,661,458 (BERRY) 28 April 1987, see column 4, line 50 to column 6, line 54 and column 8, line 14 to column 9, line 56.	1-33

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [4]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.